

Combined HLA matched limbal stem cells allograft with amniotic membrane transplantation as a prophylactic surgical procedure to prevent corneal graft rejection after penetrating keratoplasty: case report

Paolo Capozzi, Sergio Petroni and Luca Buzzonetti

Dipartimento Chirurgico, Oculistica, Ospedale Pediatrico Bambino Gesù, Rome, Italy

Abstract

Purpose. To determine if the use of combined HLA matched limbal stem cells allograft with amniotic membrane transplantation (AMT) is a safe and effective prophylactic surgical procedure to prevent corneal graft after penetrating keratoplasty (PK).

Methods. We report the case of a 17 years old patient with a history of congenital glaucoma, trabeculectomy and multiple corneal graft rejections, presenting total limbal cell deficiency. To reduce the possibility of graft rejection in the left eye after a new PK, a two step procedure was performed. At first the patient underwent a combined HLA matched limbal stem cells allograft (LAT) and AMT and then, 10 months later, a new PK.

Results. During 12 months of follow-up, the corneal graft remained stable and smooth, with no sign of graft rejection.

Conclusions. In our patient, the prophylactic use of LAT from HLA-matched donors and AMT before PK, may result in a better prognosis of corneal graft survival.

Key words

- limbal stem cells
- HLA matched allograft
- corneal transplantation
- amniotic membrane transplantation

INTRODUCTION

The corneal epithelium maintains homeostasis by balancing desquamation from the surface and proliferation of the basal cells plus centripetal movement from the stem cells located at the limbus [1]. When the corneal limbus is severely damaged (limbal stem cell deficiency, LSCD), conjunctival epithelium replaces the corneal epithelium, which causes significant visual deterioration. The surgical prognosis of penetrating keratoplasty (PK) in eyes with limbal dysfunction remains very poor, because the epithelium on the graft is not supplied by stem cells [2]. The autograft limbal stem cells transplantation was described for the first time in 1989 [3] and recently transplantation of the limbal tissues using either autografts [4-6] or allografts (limbal allograft transplantation, LAT) has been proposed for the treatment of limbal dysfunction [4].

As described by Kolli *et al.* [5], the use of stratified cultivated oral mucosa epithelium was successful in two patients with bilateral total LSCD with reversal

of the deficit up to 24 months. Zakaria *et al.* [6] described the results of a cultivated limbal stem cell protocol in a prospective phase I/II non-randomized clinical trial in 18 consecutive patients with total and partial limbal stem cell deficiency. Their standardized, non-xenogenic, reduced manipulation cultivation and surgical transplantation of limbal stem cell grafts has shown to be a safe and effective treatment option for patients with LSCD.

One major concern, however, about the autologous transplantation of limbal epithelial stem cells is the wellbeing of the donor fellow eye, from which one third to a half of the limbal circumference has been removed. So, when also the fellow eye show damage of the limbal stem cells, the allograft transplantation is the safer treatment option. The tissues used for the LAT can be obtained by living donor or eye bank eyes [4]. One important problem concerning allografts is that, in most of the cases, it is not HLA matched and so it requires prolonged, if not indefinite, immunosuppression.

An alternative surgical solution that can help to restore corneas with limbal cell defects is amniotic membrane transplantation (AMT). Tseng *et al.* [7] have demonstrated that AMT is effective in treating partial limbal stem cell defects.

We report the case of the use of combined HLA matched limbal stem cells allograft with AMT as a prophylactic surgical procedure to prevent corneal graft rejection after PK.

REPORT OF A CASE

A 17-year-old girl with a history of congenital glaucoma in both eyes had her intraocular pressure (IOP) normal after bilateral trabeculectomy and Baerveldt valve implant, performed during the first years of her life. Due to her primary ocular pathology and even if a normal IOP, she presented a bilateral corneal opacification, necessitating a bilateral PK. Postoperative examination of the anterior segment of the right eye showed an excellent result with a clear corneal graft and a final visual acuity of 20/40. Differently, the left eye presented a severe corneal edema and cloudiness. The left corneal graft condition allowed us to perform a second PK in this eye. Unfortunately also the second and then the third PK did not show acceptable postoperative results: the left corneal graft eye cornea had diffuse superficial neovascularization and stromal cloudiness. The duration of the transparency of the graft after the third PK was only eighteen days.

To reduce the possibility of graft rejection in the left eye after a new PK, a new surgical approach was taken in exam. Anterior segment examination of the left eye showed a total limbal cell deficiency and so the autologous transplantation of limbal epithelial stem cells was not performable. The HLA analysis of the patient's sister was

assessed and showed a complete HLA match. And so a two step procedure was performed. At first the patient underwent a combined HLA matched limbal stem cells allograft and AMT and then, 10 months later, a new PK.

Surgical techniques

Penetrating keratoplasty

The donor cornea was trephined with a punch and the receiving cornea with a suction trephinator. The trephination diameter in the receiving cornea is in the range of 7.5-7.75 mm, both with a donor trephination 0.25-0.5 mm larger. Viscoelastic material is placed on the surface of the receiving cornea and the graft is placed on top of it and affixed with symmetrical points (nylon 10.0) at 6 and 12 hours. Having affixed the two points, The third and the fourth point are placed at 3 and 9 hours and then the suture of the graft is completed with single sutures (*Figure 1, A-D*).

Limbal stem cells allograft and amniotic membrane transplantation

To obtain stem cell allograft, the sister's donor eye underwent a removal of 2 patches both consisting in 7 mm of conjunctiva and 2 mm of clear cornea taken at 6 and 12 hours.

One patch was obtained with manual excision, while the other using the 60 kHz IntraLase femtosecond laser (IntraLase FS Laser, Abbott Medical Optics, Inc.) loaded with the IntraLase-enabled keratoplasty computer (*Figure 1, E, F*).

Amniotic membrane was sutured on the receiving cornea with Nylon 10.0 stitches and then, above HAM, the 2 patches were sutured at 6 and 12 hours: vicryl 8.0 was used for the conjunctival stitches and nylon 10.0 for the corneal ones (*Figure 1, G*).

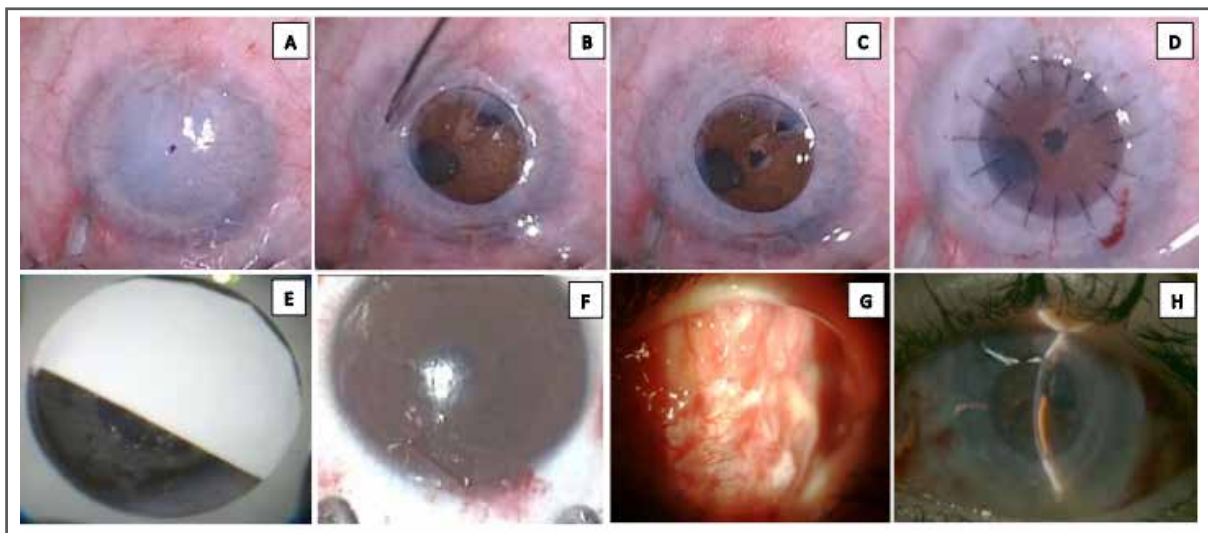


Figure 1

Legend: Preoperative clinical condition is showed in (A). After donor trephination is performed, the wide iridectomy, performed in the previous surgery for congenital glaucoma, and corectopia are visible (B). Pupilloplasty is performed (C). The corneal graft is finally sutured with single stitches (D). Laser assisted (E) and manual (F) removal of donor patches consisting in 7mm of conjunctiva and 2mm of clear cornea. In (E) the carton mask positioned over the laser lens to obtain the laser cut corneal- conjunctival patch is shown. Amniotic membrane was then sutured (G). The corneal graft of the left eye remained stable and smooth, with clear stroma and no sign of graft rejection (H).

During 12 months of follow-up, the corneal graft of the left eye remained stable and smooth, with clear stroma and no sign of graft rejection. The best-corrected visual acuity improved to 4/20. The IOP was 11 mmHg. No complications were found in the donor eye (*Figure 1, H*).

DISCUSSION

The amniotic membrane has effectively been used as a temporary patch to promote healing of the ocular surface by reducing inflammation and scar formation [8]. This healing effect lends its use in multiple cases of ocular surface pathology, including corneal ulcers, severe ocular surface desiccation from chemical and thermal burns, limbal stem cell deficiency, and scleral melt [9, 10]. Recent studies and applications of amniotic membrane provide confirmation of earlier investigations and there is mounting agreement that early intervention with amniotic transplantation in cases of severe pathology is key to preserving the ocular surface and ameliorating visual function.

The autografts or allografts has been proposed for the treatment of with LSCD [4-6]. The surgical option and postoperative success of the limbal stem cells transplantation technique depend on the health of patient's limbal stem cells (LSCs). Autograft is not applicable to patients with bilateral LSCD where there are no remaining LSCs.

Limbal allograft transplantation from a heterologous donor has been shown to be effective and safe methods of treatment for corneal surface reconstruction because of various aetiologies. The disadvantages of limbal

allografting are the risk of graft rejection and side effects of systemic immunosuppression against limbal autografting.

However, risk of graft rejection, discontinuation of immunosuppression, advanced stage ocular surface problems particularly as in these eyes, difficulty of finding HLA-matched donors, and availability of sufficient amount of graft tissue from donor eye, were all reported to worsen the surgical outcomes in these patients. We presume that the success of our case is due to the rarity of finding a 100% HLA matched donor, avoiding systemic immunosuppression. Despite its capacity of improving graft survival, systemic immunosuppression has an important morbidity being a long lasting therapy.

In conclusion, LAT from HLA-matched donors combining it with penetrating keratoplasty may result in a better prognosis of graft survival and improved visual function in these eyes.

Even if we realize that it is not possible to extrapolate a general guideline from 1 case report, our success warrants others to consider such an option when considering the risk in the donor eye during the treatment of unilateral LSCD.

Conflict of interest statement

The authors have no financial or proprietary interests in this study.

Received on 10 December 2013.

Accepted on 2 July 2014.

REFERENCES

1. Cotsarelis G, Cheng S-Z, Dong G. Existence of slow-cycling limbal epithelial basal cells that can be preferentially stimulated to proliferate: implications on epithelial stem cells. *Cell* 1989;57:201-9. DOI: 10.1016/0092-8674(89)90958-6
2. Buxton JN. Indication and contraindications. In: *Corneal surgery: theory, technique, and tissue*. St Louis (MO): Brightbill FS eds.; 1993. p 77-8.
3. Kenyon KR, Tseng SC. Limbal autograft transplantation for ocular surface disorders. *Ophthalmology* 1989;96(5):709-22. DOI: 10.1016/S0161-6420(89)32833-8
4. Tsubota K, Toda I, Saito H, Shinozaki N, Shimazaki J. Reconstruction of the corneal epithelium by limbal allograft transplantation for severe ocular surface disorders. *Ophthalmology* 1995;102:1486-96. DOI: 10.1016/S0161-6420(95)30841-X
5. Kolli S, Ahmad S, Mudhar HS, Meeny A, Lako M, Figueiredo FC. Successful application of *ex vivo* expanded human autologous oral mucosal epithelium for the treatment of total bilateral limbal stem cell deficiency. *Stem Cells* 2014 Mar 2. DOI: 10.1002/stem.1694
6. Zakaria N, Possemiers T, Dhubbghaill SN, Leysen I, Rozema J, Koppen C, Timmermans JP, Berneman Z, Tassignon MJ. Results of a phase I/II clinical trial: standardized, non-xenogenic, cultivated limbal stem cell transplantation. *Journal of Translational Medicine* 2014;12:58. DOI: 10.1186/1479-5876-12-58
7. Tseng SC, Prabhasawat P, Barton K, Gray T, Meller D. Amniotic membrane transplantation with or without limbal allografts for corneal surface reconstruction in patients with limbal stem cell deficiency. *Arch Ophthalmol* 1998;116:431-41. DOI: 10.1001/archoph.116.4.431
8. Tseng S. Amniotic membrane transplantation for ocular surface reconstruction. *Biosci Rep* 2001;21:481-9
9. Meller D, Pires RTF, Tseng SCG. *Ex vivo* preservation and expansion of human limbal epithelial progenitor cells by amniotic membrane. *Invest Ophthalmol Vis Sci* 1999;40: S329.
10. Taylor RJ, Wang MX. Rate of re-epithelialization following amniotic membrane transplantation. *Invest Ophthalmol Vis Sci* 1998;39:S1038.